**Title:** Inferring pesticide toxicity to honey bees from a field-based feeding study using a colony dynamics model and Bayesian inference

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**Target journal:** Ecological Applications

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**Abstract**

Honey bees are crucial pollinators for agricultural crops but are threatened by a multitude of stressors including exposure to pesticides. Linking our understanding of how pesticides affect individual bees to colony-level responses is challenging because hives show emergent properties based on complex internal processes and interactions among individual bees. Agent-based

models that simulate honey bee colony dynamics may be a tool for scaling between individual and colony effects of a pesticide. The U.S. Environmental Protection Agency (USEPA) and U.S. Department of Agriculture (USDA) are developing the VarroaPop+Pesticide model which simulates the dynamics of honey bee colonies and how they respond to multiple stressors, including weather, varroa mites and pesticides. To evaluate this model, we used Approximate Bayesian Computation to fit field data from an empirical study where honey bee colonies were fed the insecticide clothianidin. This allowed us to reproduce colony feeding study data by simulating hive demography and mortality from ingestion of contaminated food. We found that VarroaPop+Pesticide was able to fit general trends in colony population size and structure and reproduce colony declines from increasing clothianidin exposure. Model predictions of the lowest observed adverse effect concentration (LOAEC) fell within a factor of 2 of the LOAEC observed in the empirical data. The model underestimated adverse effects at low exposure (36 µg/kg), however, and overestimated recovery at the highest exposure level (140 µg/kg), for the adult and pupa endpoints, suggesting that mechanisms besides oral toxicity-induced mortality may have played a role in colony declines. The VarroaPop+Pesticide model estimates an adult oral $LD_{50}$ of 16.3 ng/bee (95% CI: 10.2–26.6) based on the simulated feeding study data, which falls within the 95% confidence intervals of values observed in laboratory toxicology studies on individual bees. Overall, our results demonstrate a novel method for analyzing colony-level data on pesticide effects on bees and making inferences on pesticide toxicity to individual bees.

## Introduction

Honey bees (*Apis mellifera* L.) provide essential pollination services for many agricultural crops, but these services are threatened by increasing colony losses in North America and Europe in

48 recent decades (Tylianakis 2013, Potts et al. 2016). While multiple stressors (disease, nutrition,
49 genetics and chemicals) are implicated, pesticides may be important contributors to these
50 declines (National Honey Bee Health Stakeholder Conference Steering Committee 2012,
51 Goulson et al. 2015) because they can cause direct mortality to individual bees, as well as a
52 range of sublethal effects (Krupke et al. 2012) and have been found in hives (Mullin et al. 2010).
53 Linking the effects of pesticides on individual bees to whole-colony success or failure is
54 challenging because hives are complex systems (*i.e.*, superorganisms) with emergent properties
55 derived from internal population dynamics and complex interactions among individuals (Seeley
56 1995, Camazine et al. 2003, Godfray et al. 2014). While it is possible to measure declines in
57 colony-level properties over time (*e.g.*, number of adult bees and cells of honey), it is difficult to
58 observe effects of pesticides inside hives at the individual bee level and directly link individual-
59 level and colony-level effects. Agent-based models that simulate internal hive population
60 dynamics in response to pesticide exposure may allow inference on how pesticide effects on
61 individual bees scale up to colony growth and survival.
62 Many managed honey bee colonies are located in or near agricultural areas, leading to
63 exposure to pesticides being applied to control crop pests (Mullin et al. 2010, Tosi et al. 2018).
64 Two primary routes of pesticide exposure for honey bees have been identified: contact and diet.
65 Contact exposure with pesticides occurs when foraging bees are directly sprayed or when they
66 land on foliage that has received direct spray or drift (Girolami et al. 2012, Krupke et al. 2012).
67 Dietary exposure occurs through ingestion of pollen or nectar derived from either pesticide-
68 treated agricultural crops (Girolami et al. 2009, Krupke et al. 2012) or from neighboring wild
69 plants contaminated through drift or transfer through soil and subsequent root uptake (Krupke et
70 al. 2012, USEPA et al. 2014, Bonmatin et al. 2015, Mogren and Lundgren 2016, Botías et al.

2016). Furthermore, some classes of pesticides are relatively stable in the environment, leading to soil contamination that can persist for months or years after application (Goulson 2014, Jones et al. 2014). Acute and chronic pesticide exposure to honey bees can lead directly to bee mortality, or indirectly via sublethal effects such as reduced foraging success (Henry et al. 2012), impaired olfactory learning (Decourtye et al. 2005), and increased susceptibility to diseases (Alaux et al. 2010, Di Prisco et al. 2013, Doublet et al. 2015).

Over the past decade, regulatory agencies such as the U.S. Environmental Protection Agency (USEPA) and European Food Safety Authority (EFSA) have developed guidance for risk assessors and stakeholders on data needs for honey bee toxicity testing, as well as how to evaluate of potential risk of pesticides to bees (e.g., EFSA 2013, USEPA et al. 2014, Rortais et al. 2017). These efforts were partly a response to public concern over significant losses of honey bee colonies in the US and Europe (National Research Council 2007, Oldroyd 2007), and also due to development of increasingly reliable laboratory toxicity testing protocols for honey bees.

USEPA's process for assessing risk to bees utilizes a tiered approach that begins with acute and chronic testing of individual adult and larval honey bees, and in higher tiers, considers exposures and effects to colonies. In Tier I toxicity studies, individual larval or adult bees are exposed to a single contact or oral dose (acute toxicity studies) or repeated oral doses (chronic toxicity studies) of a given pesticide. These studies derive standard toxicity endpoints based on apical endpoints (survival, growth or reproduction) that can be compared to estimated environmental exposures. Acute exposure endpoints based on mortality are represented by median lethal doses ($LD_{50}$ values), while chronic exposure toxicity endpoints are represented by Lowest Observed Adverse Effect Concentrations (LOAECs) and No Observed Adverse Effect Concentrations (NOAECs). These values are compared to Estimated Environmental

Concentrations (EECs) for contact and oral exposures of a given pesticide that are generated using the BeeREX model (USEPA et al. 2014). These comparisons, represented by risk quotients (RQs), are then compared to the Levels of Concern (LOCs) for acute and chronic toxicity, 0.4 and 1.0, respectively, which were established by the USEPA to define whether there is a potential risk concern for effects to individual bees (USEPA 2012).

Based on the results of Tier I studies, Tier II testing may be conducted. Tier II studies involve comparison of empirically-based concentrations of pesticides in pollen and nectar to results of controlled colony-level toxicity studies (colonies are fed known concentrations of a pesticide), as well as consideration of effects to hives exposed in semi-field conditions (tunnel or enclosure studies). If there are risk concerns (adverse colony-level effects at empirically-observed concentrations of pesticides) from the more controlled Tier II studies, full-field (Tier III), colony studies may be needed (USEPA et al. 2014). Semi- and full-field studies evaluate pesticide toxicity at the colony-level, including potential measurement of adverse effects on sublethal honey bee behavior such as foraging activity, and quantification of toxicity effects on honey bee brood and food production. Higher-tier studies are considered more representative of real field exposures by honey bee colonies, but interpretation of their data can be confounded by interactions with other environmental influences and stressors (*e.g.*, disease, nutrition, and parasites) and variability among hives, and they are logistically challenging and expensive to conduct (USEPA et al. 2014). Models are therefore being developed in the US and EU to simulate colony-level effects of pesticides to aid synthesis of colony-level data, and to provide regulatory agencies with additional evidence of whether higher tier (Tiers II or III) studies may be informative.

The US model under development is VarroaPop+Pesticide (hereafter, VarroaPop), an age-structured, agent-based colony simulation model (Kuan et al. 2018). It was first developed to simulate colony growth and development through time (BEEPOP; DeGrandi-Hoffman et al. 1989), and subsequently extended to include infestation by parasitic Varroa mites (*Varroa destructor*) (Degrandi-Hoffman and Curry 2005) and pesticide exposure (Kuan et al. 2018) to determine their cumulative effects on colony growth and survival. Pesticide contamination of pollen and nectar can be calculated based on application method, similarly to the Tier 1 BeeREX model, or directly specified. Individual food consumption rates for each age and caste of bees are used to scale up exposure to the entire hive. Toxicity is applied to each day-cohort, based on the logistic Hill equation with $LD_{50}$ and slope parameters (Hill 1910).

Here, we present a method for inferring individual-level pesticide toxicity from colony-level field data, employing the VarroaPop+Pesticide agent-based colony model. We used data from a registrant-submitted feeding study on clothianidin, a nitroguanidine-substituted neonicotinoid insecticide, in which hives were dosed with spiked nectar of varying concentrations over a five-week period. Because nectar contaminated with the active ingredient was provided directly to hives, this study focuses only on dietary exposure routes. We implemented a Bayesian hierarchical model based on VarroaPop to explain dynamics of single colonies in the feeding study. We then applied Approximate Bayesian Computation (ABC) to fit our model to the empirical data and inferred parameters describing individual toxicity in VarroaPop. We hypothesized that (1) VarroaPop can explain general trends in colony population size observed in the control hives and (2) individual-level oral toxicity is sufficient to explain colony declines observed at high concentrations of clothianidin in the feeding study.

## Methods

*Colony feeding study design*

A study on the effects of clothianidin on honey bee colonies in field conditions took place between June 17, 2014 and April 27, 2015 in the Piedmont region of North Carolina. Eighty-four hives were divided into twelve sites in low-agriculture areas of Guilford, Randolph, Alamance and Chatham counties. Hives were assigned to one of six treatment groups that received supplemental nectar feedings spiked with clothianidin at 0, 10, 20, 40, 80 and 160 µg/L. Measured clothianidin concentrations in the supplemental nectar were found to be slightly lower than the nominal treatment levels, so we refer to the treatments by the measured values, in units of µg/kg: 0, 10, 19, 36, 72 and 140 µg/kg. We also used these measured values as the pesticide inputs to VarroaPop. Clothianidin concentrations observed in crop nectar following foliar, soil and seed applications range from 4–3400 ng/g, 4–40 ng/g and 1–4 ng/g, respectively, and represent a variety of locations, conditions and crops (USEPA 2017). Treatments were assigned with a stratified random approach that standardized colony size among the treatments. There were 24 replicate colonies for the 0 µg/kg control, and 12 replicates for each of the five treatment levels. Supplemental nectar feeding occurred continuously for 34 days from June 26, 2014 to July 30, 2014, with clothianidin content prescribed by treatment level. However, we refer to the nominal treatment values in the text, to be consistent with the language used in the colony feeding study. In addition to the supplied nectar, bees were allowed to forage naturally for pollen. All hives were treated for *Varroa* mites with an application of thymol in September 2014 in accordance with typical apicultural practice for the region (Louque 2016).

The condition of each colony was assessed before nectar feedings began (June 18-23), once during the feedings (July 15-18), and one, five, and eleven weeks post-feeding (August 5-

11, September 8-12 and October 14-22, respectively). During each colony condition assessment, hives were opened, and each frame was removed and inspected. Area coverage was measured for adult bees, larvae, pupae, eggs, honey, nectar, and pollen (bee bread). Area measurements were then converted to individual or cell counts, using density of adult bees (for adults) or density of cells on the frame (for all other endpoints) empirically measured in the study. To parameterize the initial nectar and pollen store parameters in VarroaPop+Pesticide, we converted nectar and pollen cell counts to weight, using cell depth of 12.5 mm, nectar density of 1.13 g/ml (30% sucrose solution), and pollen density of 1.45 g/ml (corn pollen, a major pollen source for the study hives) (Aylor 2002). Data used in our analysis (replicate-level means and standard deviations) are publicly available (Louque 2016).

*VarroaPop+Pesticide model*

The complete structure and equations of VarroaPop are described elsewhere (DeGrandi-Hoffman et al. 1989, DeGrandi-Hoffman and Curry 2004, Degrandi-Hoffman and Curry 2005, Kuan et al. 2018); here, we provide a brief summary. VarroaPop is an agent-based model that simulates colony dynamics, based on queen egg-laying rate, development of workers and drones, and activity patterns of foragers. These dynamics are optionally modified by *Varroa* mite infestation (not considered here), and oral and/or contact exposure to pesticides. Queens are simulated as individual agents, with daily egg-laying rate and proportion of eggs fertilized determined by weather, colony size, worker population, photoperiod and fecundity (queen strength) (DeGrandi-Hoffman et al. 1989). All other bees are simulated as day-cohort agents which age and transition between life stages and consume pollen and/or nectar based on age and caste (Rortais et al. 2005, USEPA 2014).

We focused our modeling on a time window spanning just before nectar feeding treatments began until eleven weeks post-treatment. VarroaPop+Pesticide requires daily weather data on temperature, precipitation, hours of daylight, and wind speed for the simulation period to determine potential foraging time. We used National Oceanic and Atmospheric Administration (NOAA) weather data gridded at 0.25° x 0.25° resolution and centered at 35.875° N, 79.375° W near the center of the feeding study area (Fry et al. 2016). Mean daily temperature during the exposure period was 23.3 C, close to the 15-year average for this period of 23.9 C. Mean daily precipitation was 0.24 cm/day, 33% lower than the 15-year mean of 0.36 cm/day. In the model, when weather is favorable (maximum temperature between 12 C and 43.3 C, wind speed less than 21.1 m/s, daily rainfall less than 0.5 cm), foraging honey bees collect pollen and nectar from an infinitely large range area, based on a specified number of trips per day. Resources collected in excess of daily consumption are stored and potentially consumed later when daily foraging does not meet hive food requirements. Pesticide contamination of pollen and nectar can be calculated based on application method and timing, or directly specified (as in this study) (Kuan et al. 2018). Mortality due to ingestion of this contaminated pollen/nectar is calculated for larval and adult age-cohorts, based on the dose consumed and a logistic dose-response curve (parameterized by $LD_{50}$ and slope) (Hill 1910, Kuan et al. 2018). Contact exposure to foraging bees can also be simulated in pesticide foliar spray scenarios, but is not considered in this study, which included only dietary exposure.

*Modeling the feeding study data using VarroaPop+Pesticide*

We defined a Bayesian hierarchical model, which included the VarroaPop+Pesticide agent-based model, to explain dynamics of single colonies in the feeding study. We then used it to simulate

the 84 colonies in the feeding study and produce treatment by time point summary statistics that corresponded to observations in the study. We modeled the population structure of an individual colony $i$ at a given time point $t$ ($\boldsymbol{y}_{i,t}$) as

$$\boldsymbol{y}_{i,t} = f(\boldsymbol{y}_{i,0}, \boldsymbol{x}, \boldsymbol{z}, Init_i, E_i, Str_i, L_i, t) \quad (1)$$

$$Str_i \sim Normal(\mu_{Str}, \sigma_{Str})$$

$$L_i \sim Normal(\mu_L, \sigma_L)$$

$$\boldsymbol{x}_j \sim Unif(a_j, b_j) \text{ for } j=1,2,\ldots,n$$

$$\mu_{Str,L} \sim Unif(a^{\mu}_{Str,L}, b^{\mu}_{Str,L})$$

$$\sigma_{Str,L} \sim Unif(a^{\sigma}_{Str,L}, b^{\sigma}_{Str,L})$$

where $f$ is the VarroaPop+Pesticide agent-based model; $\boldsymbol{y}_{i,0}$ is initial population structure for colony $i$;, $\boldsymbol{x}$ is a vector of toxicity random variables; $\boldsymbol{z}$ is a vector of fixed variables including weather conditions; $Init_i$ is a vector of initial size, population structure, and food resources for colony $i$; $E_i$ is the clothianidin exposure level for colony $i$; and $Str_i$ and $L_i$ are random variables for queen strength (egg-laying rate) and forager lifespan, respectively, for colony $i$. We considered $Str_i$ and $L_i$ to be random variables drawn from a normal distribution shared among all colonies in the study because they strongly influence population dynamics and vary between colonies (Kuan et al. 2018). Thus, our model uses these two random variables to account for the variance among replicate hives in the feeding study. For the mean ($\mu$) and standard deviation ($\sigma$) hyperparameters of the normal distributions, we defined uniform hyperpriors with $\mu$ bounded within [1, 5) for queen strength (equivalent to 1000 to 3000 eggs/day) and [4, 16) days for forager lifespan, the full range of possible values in VarroaPop, and $\sigma$ within [0, 2) and [0, 3), respectively (Table 1). We also defined prior probability of toxicity parameters $\boldsymbol{x}$ as a uniform distribution spanning the range of plausible values (Table 1). Adult and larva oral $LD_{50}$ was

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231 defined within [0.1, 100) ng/bee, a considerably wider range than that observed in laboratory
232 studies (USEPA 2017). Adult and larva dose-response curve slope was defined within [1, 9) %
233 mortality per ng clothianidin, the range defined in a previous sensitivity analysis of VarroaPop
234 (Kuan et al. 2018). We then used Bayesian inference to estimate the joint posterior probability of
235 toxicity parameters $\boldsymbol{x}$ and hyperparameters $\mu_{\mathrm{Str}}$, $\sigma_{\mathrm{Str}}$, $\mu_{\mathrm{L}}$ and $\sigma_{\mathrm{L}}$.

236 In addition to initial hive conditions, weather data, and toxicity parameters,
237 VarroaPop+Pesticide requires parameterization of pollen and nectar foraging behavior and
238 consumption rates for each life stage. We treated these as known constants shared among all
239 colonies in the feeding study (Appendix 1 Table S1). For pollen and nectar consumption rates,
240 we used the empirically-derived values compiled in the USEPA Final Guidance on Bee Risk
241 Assessments document, taking the mean when ranges were given (USEPA 2014). For the
242 number of nectar-gathering trips per day, we started with 10/day, the mean value used by the
243 USEPA Bee Risk Assessment Framework document (USEPA 2012) and increased this until
244 nectar stores could be maintained in VarroaPop for control treatment hives. This resulted in a
245 final value of 17 trips/day, which is within the previously reported range for foraging honey bees
246 (Winston 1987). Because pollen foraging occurs primarily during the first half of the day, we set
247 the number of pollen trips to 8/day which is close to the previously reported mean pollen
248 foraging activity by honey bees (Klein et al. 2019).

249 To confirm that our model could fit the general population structure of the control hives,
250 we did an initial VarroaPop+Pesticide run with the mean initial conditions of the control and
251 previously described parameters. We observed that, although the adult population count
252 estimated by the model was in agreement with empirical data from the study, there were more
253 pupae, larvae and eggs in the empirical data than was predicted by VarroaPop, suggesting

254 unexplained mortality as pupae transition to adults. We therefore reduced the pupa-to-adult
255 transition survival rate in the model from 100% to 60% (Appendix 1 Table S1), the level at
256 which the predicted population structure roughly matched the control data.
257
258 *Model inference using Approximate Bayesian Computation with sequential Monte Carlo*
259 We used Approximate Bayesian Computation (ABC) to infer probability distributions of toxicity
260 parameters in our model, given the empirical feeding study data. ABC is a computational method
261 for approximating the joint posterior probability distribution of a model by comparing its
262 outcome to empirical data (either with individual data points or summary statistics) (Beaumont
263 2010, Csilléry et al. 2010). We compared the mean and standard deviation of empirical and
264 estimated colony endpoints (number of adults, pupae, larvae and eggs) for each treatment group
265 by time combination. Parameter sets (particles) are either accepted or rejected based on whether
266 their distance from the real data, as calculated by a distance function on summary statistics (in
267 this case the sum of absolute deviation), is less than an acceptance criterion $\epsilon$. A key advantage
268 of ABC is that this distance function replaces a formal likelihood function, allowing inference on
269 black-box or agent-based models like VarroaPop which lack a tractable likelihood function.
270 To explore parameter space and propose potential parameter sets for ABC, we used a
271 sequential Monte Carlo (SMC) algorithm, also known as particle filtering (Sisson et al. 2007,
272 Toni et al. 2009, Doucet and Johansen 2011). This algorithm uses Monte Carlo iterations (called
273 populations), each of which takes the distribution of particles accepted by ABC in the last
274 population as the prior distribution from which to sample. With each successive population, the
275 acceptance criterion $\epsilon$ is decreased, resulting in an increasingly close approximation of the
276 posterior.

277 To carry out ABC with SMC sampling (ABC-SMC), we used the pyABC package
278 version 0.9.2 in Python 3.6 (Klinger et al. 2018), with computation distributed across 40 cores.
279 We used the sum of absolute deviation ($L_1$ norm) as the distance function because it may be
280 more robust to outliers than the commonly-used sum of squared deviations ($L_2$ norm), but
281 performs similarly, overall (Prangle 2017). We chose to fit our model to the mean and standard
282 deviation of adult and egg counts because these two endpoints should provide sufficient
283 information to estimate all intermediate life stages. Thus, our distance function compared model
284 predictions and empirical data for 96 summary statistics (6 treatments x 4 dates x 2 endpoints x 2
285 statistics). For the transition function, which converts each set of accepted particles to the prior
286 for the next generation, we chose a local multivariate gaussian kernel density estimator (KDE),
287 using the nearest quarter of neighbors, which leads to faster convergence than a global KDE
288 (Filippi et al. 2013). We adjusted $\epsilon$ each generation to the median distance of accepted particles
289 in the prior generation. We used a population size of 500 accepted particles for the first 11
290 populations; for populations 12 and 13, we reduced the population size to 100, due to the
291 computational demands of finding accepted particles when epsilon is very low. We ended
292 sampling after 13 populations because computational time had become prohibitive for such small
293 return, since $\epsilon$ decreased only slightly with each additional generation and marginal posterior
294 distributions had become stable.
295
296 *Predicting colony response to clothianidin*
297 We used our fitted model to make predictions by sampling the joint posterior, which involved
298 drawing parameters from our final generation of accepted particles -- weighted by their distance
299 from the empirical data -- and evaluating the model using each parameter set to produce

300 synthetic feeding study data. After 200 samples from the posterior, resulting in 200 model
301 evaluations, we calculated the median value for the prediction of interest, as well as the $2.5^{th}$ and
302 $97.5^{th}$ percentiles to create 95% prediction intervals. These reflect variation in individual colony
303 strengths, as well as clothianidin toxicity parameters, as inferred from the empirical data.
304 Because we sampled from the joint posterior, all predictions reflect the covariance structure of
305 the parameters.
306 We used this method to predict the clothianidin adult and larva oral dose-response curves,
307 and distribution of egg-laying rates (derived from queen strength) and forager lifespans among
308 colonies in the feeding study. We also predicted colony population structures through time for
309 each treatment and compared these results to predictions for the control treatment. We then
310 assessed whether our model predicted a significant reduction in the number of adults, pupae,
311 larvae and eggs at any time during the study, for each treatment level, as well as several untested
312 exposure levels between the NOAEC and LOAEC (36 µg/kg and 72 µg/kg, respectively)
313 observed in the feeding study. We defined a significant reduction as a period when the predicted
314 difference from the control was significantly below zero (lower 95% prediction interval did not
315 contain zero).
316
317 **Results**
318 *Details of ABC-SMC sampling*
319 We used Approximate Bayesian Computation with sequential Monte Carlo sampling (ABC-
320 SMC) to infer posterior probability distributions of key parameters in our VarroaPop+Pesticide-
321 based statistical model. Sampling occurred over 13 populations, with acceptance rates that began
322 at 51.9% and decreased to 1.3% (Figure 1). The total number of parameter sets (particles)

considered was 62,346, each of which required 82 individual runs of VarroaPop+Pesticide, for a total ~5.1 million model runs. Actual computation time was approximately 38 days. Sampling was stopped after population (SMC iteration) 13 due to increasingly long computation times yielding little improvement in the acceptance threshold $\epsilon$ (Figure 1). At this point in the algorithm, there were no major shifts in the posterior probability of parameters between generations (Figure 2).

*Comparison of model predictions to the empirical feeding study data*

To assess whether our VarroaPop+Pesticide-based model could explain patterns of the feeding study, we compared our ABC-SMC-parameterized model's predicted colony demographics through time to empirical data. For control treatment hives, model predictions matched general temporal trends which included a relatively stable adult population, sudden declines in pupae and adults at the final sampling point, and a consistent decrease in the number of eggs (Figure 3; also see Appendix 2 Figure S2). The model also successfully predicted declines across all population endpoints (counts of each caste) for the 72 and 140 µg/kg treatments, although it underpredicted the magnitude of decline at 140 µg/kg for adults, pupae and larvae. Our 95% prediction intervals, which captured variability in parameter values and individual hive strength, overlapped with the standard deviation of the field data in 20/24 treatments (83.3%) by sampling date combinations, excluding the initial time points.

*Predicted no/lowest observed adverse effect concentration (NOAEC/LOAEC)*

We used our predicted colony size and population structure trajectories across the clothianidin treatments to estimate the no observed adverse effect concentration (NOAEC) and lowest

346 observed adverse effect concentration (LOAEC) for adult, larvae, pupae and egg endpoints.
347 When comparing all treatment levels present in the colony feeding study to the control, our
348 model predicted a NOAEC and LOAEC of 36 and 72 µg/kg, respectively, on the basis of adverse
349 effects on adults and brood (Figure 4, Appendix 2 Figure S3). Across endpoints, colonies in the
350 72 µg/kg treatment had significant population reductions versus the control for 46.8% of the
351 study period, on average (*i.e.*, 46.8% of the time the 95% prediction intervals for change in
352 number bees from the control did not contain zero; Table 2). Bee colonies in the 140 µg/kg
353 treatment were affected more severely, with significantly lower populations than the control for
354 88.7% of the study period, on average (Table 2).
355 Our parameterized model also allowed us to estimate a more precise NOAEC and
356 LOAEC (between 36 and 72 µg/kg) than was possible in the colony feeding study, by predicting
357 adverse effects at intermediate treatment levels that were not included in the feeding study
358 design. We predicted effects on colonies from exposure to 50, 55, 60, 65 and 70 µg/kg
359 clothianidin-spiked nectar, and found an estimated NOAEC and LOAEC of 55 and 60 µg/kg,
360 respectively, based on adverse effects on the number of adult bees (Figure 5), and brood
361 (Appendix 2 Figure S4). Across all endpoints, colonies exposed to 55 µg/kg clothianidin had
362 significant population reductions versus the control for only 2.8% of the study period on average,
363 an effect which may not be biologically significant for colony survival. In contrast, colonies
364 exposed to 60 µg/kg clothianidin had significant adverse effects for 33.6% of the study period,
365 on average (Table 2).
366
367 *Probability distributions of model parameters inferred from feeding study data*

Through ABC-SMC, we inferred the most probable parameters for our model from empirical feeding study data. We considered four VarroaPop+Pesticide parameters that characterize pesticide toxicity at the individual bee level. Adult $LD_{50}$, the median lethal oral dose for adults and foragers, had a large impact on our model's ability to fit the empirical data and, therefore, had a sharply defined marginal posterior probability distribution (Figure 6). The median adult $LD_{50}$ was 16.3 ng/bee, with a 95% credible interval (CI) of 10.2–26.6 ng/bee (Figure 2). The slope of the adult oral dose-response curve had a median value of 6.1 (95% CI: 1.5–8.7). In contrast with adult oral toxicity, larval toxicity did not show a strong marginal trend. The median larval $LD_{50}$ was 62.4 ng/bee, with a 95% CI that covered most of the possible range (7.3 –98.0 ng/bee), and median slope of the larva dose-response curve was 5.74 (95% CI: 1.5–8.6).

We also inferred population-level parameters that described distribution of colony strength across hives in the feeding study (Figure 6). Queen strength, which controls the maximum egg-laying rate in VarroaPop and varies from 1 to 5, had a mean of 3.6 (95% CI: 2.7–4.8) and a standard deviation of 1.8 (95% CI: 1.4–2.0). Forager lifespan, which varies from 4 to 16 days in VarroaPop, had a mean of 14.5 days (95% CI: 13.0–15.9 days) and a standard deviation of 1.0 days (95% CI: 0.1–2.7 days).

*Model predictions of clothianidin toxicity and colony strength*

We used our parameterized model to infer clothianidin dose-response curves that best explain the empirical feeding study data. The median adult oral dose-response curve indicated that individual mortality, at a rate of at least 1%, began at 5.2 ng/bee and increased to 99% by 34.8 ng/bee (Figure 7, left). Accounting for uncertainty in the adult $LD_{50}$ and slope parameters, 95% of dose response curves exhibited at least 1% mortality at a dose of less than 11.4 ng/bee and

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391 reached at least 80% mortality by 52.3 ng/bee. The larva oral dose-response curve was more
392 variable due to greater uncertainty in the larva $LD_{50}$ and slope parameters (Figure 7, right). The
393 median larva curve exhibited a mortality rate of at least 1% at 28.1 ng/bee and reached 93.7% at
394 100 ng/bee. Considering the variability in larva dose-response curves, 95% of curves showed at
395 least 1% mortality at a dose of less than 42.6 ng/bee and at least 50.2% mortality 100 ng/bee.
396 We also inferred distributions of queen egg-laying rates and forager lifespan among
397 individual colonies, by sampling from the posteriors of the population-level means and standard
398 deviations. Queen egg-laying rates varied widely from 1000 to 3000 eggs/day, but most fell
399 between 1750 and 2500 eggs/day (Appendix 1 Figure S1: left). In contrast, the distribution of
400 forager lifespan among colonies was tightly concentrated between 13 and 15 days (Appendix 2
401 Figure S1: right).
402
403 **Discussion**
404 Our study demonstrates that the VarroaPop colony simulation model can be successfully fit to
405 empirical field data from colony-level toxicity studies, providing novel inference on in-hive
406 dynamics. Because field-based colony-level studies are logistically and financially expensive,
407 models like VarroaPop are a promising method for gaining additional information on colony-
408 level effects using input parameters from laboratory toxicity testing. Furthermore, colony
409 simulation models can help separate effects of pesticides from factors like weather, temporal
410 shifts in demography (e.g., population growth/reduction and change in structure), and hive-to-
411 hive variation in queen egg-laying rate. Our analysis of the clothianidin feeding study data
412 suggests that acute oral toxicity to adult workers and foragers is sufficient to explain the majority

of colony declines observed at 72 and 140 µg/kg, although additional mechanisms appeared to prevent population recovery at 140 µg/kg.

*Simulating honey bee colony population trends and structure with VarroaPop*

The VarroaPop model, when parameterized to the feeding study data using ABC, was able to predict overall trends in colony population through time as well as general caste structure, supporting our hypothesis that the model could reproduce general trends in the data. Trends in control data that were predicted included an initial increase in the number of adult bees; a decline in the number of adults, pupae and larvae at the last time point; and a consistently decreasing number of eggs. Although VarroaPop fit overall data trends, there was a consistent deviation from empirical results. VarroaPop predicted an initial spike and recovery in the predicted adult and pupa populations, causing them to peak several weeks earlier than in the feeding study. This lagged response error is likely caused by initialization behavior of the VarroaPop program which distributes all bees within each caste evenly, across all ages. In the feeding study, colony size was increasing at the beginning of the study period and it is likely that most bees were at the young end of their age ranges, leading to a later demographic peak as a function of pupae and adult development in the empirical data relative to the model (Page and Peng 2001). This behavior may be alleviated by allowing uneven distributions of bees across age ranges, or by obtaining data for a sufficiently long pre-treatment period that allows the model to equilibrate to a natural age distribution based on egg-laying-rate.

The VarroaPop model also fit the general caste structure of the colonies in the feeding study. Both the empirical data and model predictions had a ratio of non-forager adults:pupae:larvae around 2:2:1 for control hives at all time points, except the final one.

436 Interestingly, this ratio of non-forager adults is 35–75% lower than the range predicted by a
437 steady-state model bee population using mortality rates from the literature (Torres et al. 2015).
438 To fit this low number of adult bees, we reduced the VarroaPop pupa-to-adult transition survival
439 rate to 60% from the default 100%. Lower adult bee population, relative to pupae and larvae,
440 was likely caused by background mortality from sources other than clothianidin exposure.
441 One potential source of background mortality is infection by a honey bee pathogen (e.g.,
442 chalkbrood, foulbrood, or sacbrood virus) that may kill bees at the pupal stage (Aronstein and
443 Murray 2010, Evans and Schwarz 2011), resulting in capped cells that may be counted in a
444 census but fail to produce adults. While the authors of the colony feeding study did not observe
445 these diseases (Louque 2016), they cannot be ruled out because hives were not treated for any
446 pathogens except *Varroa* mites. The authors did observe and quantify *Nosema* infection across
447 all treatment hives, however, and this pathogen causes reduced adult lifespan (Martín-Hernández
448 et al. 2011). Additionally, despite treatment for *Varroa* mites, *Varroa* presence was observed in
449 study hives, albeit at relatively low levels (0.71–2.40 mites per 100 bees in August 2014)
450 (Genersch et al. 2010). Mortality due to pathogen infection, in combination with *Varroa*
451 pressure, may have contributed to poor overwintering success following the exposure period, that
452 was noted in all treatments, including the control (Higes et al. 2008, Barron 2015).
453
454 *Using VarroaPop to explain the effect of clothianidin on colony endpoints*
455 The VarroaPop model predicted declines in each colony-level endpoint for the 72 and 140 µg/kg
456 clothianidin treatments, with magnitudes similar to those in the feeding study. The model also
457 predicted a subsequent recovery in the number of adults and pupae for these treatments to levels
458 similar to the control colonies by the final colony condition assessment (11 weeks after exposure

ended). This period of recovery in colony strength was also observed in empirical data for the 72 µg/kg hives but not the 140 µg/kg hives, which continued to decline through the end of the study period. Hives in the feeding study also exhibited significant (relative to the control), but transient, adverse effects at 36 µg/kg for two of the four endpoints considered (adults and pupae), but our model did not predict this. Taken together, our results do not support our hypothesis that ingestion-based toxicity is sufficient to explain colony declines in the clothianidin feeding study. Our model, which considered only acute oral toxicity, explained most of the negative effects seen in the empirical data and estimated a LOAEC of 60 µg/kg, within a factor of 2 of the empirical value of 36 µg/kg. However, additional mechanisms may have contributed to declines at lower exposure levels (36 µg/kg) and lack of recovery at higher exposure levels (140 µg/kg).

Our model may have underestimated effects of 36 µg/kg clothianidin spiked nectar because it did not consider chronic, sublethal effects. Based on our estimated nectar consumption values (USEPA et al. 2014), 36 µg/kg translates to a daily exposure of 13.3%–26.6% of the inferred mean $LD_{50}$ (16.3 ng/bee) for adult workers, with some variation due to age. There is a growing understanding that prolonged exposure to neonicotinoid insecticides at concentrations below lethal doses can cause adverse effects in individual bees that could ultimately affect colony performance (Godfray et al. 2015). Sub-lethal exposure appears to inhibit immune response (Brandt et al. 2016) and may lead to greater susceptibility to pathogens (Di Prisco et al. 2013, Doublet et al. 2015) including *Nosema* (Alaux et al. 2010), a unicellular parasite observed across all treatments in the feeding study. In addition, sub-lethal doses may reduce foraging success (Yang et al. 2008) by inhibiting learning and memory (Decourtye et al. 2004), navigation (Stanley et al. 2015), and locomotor function (Williamson et al. 2014, Tosi et al. 2018). Future modeling efforts could include these sublethal effects pathways, in combination with *Varroa*

mite pressure, to test whether addition of these mechanisms allows better prediction of transient colony declines at lower pesticide exposure levels.

Sublethal effects may also explain the lack of recovery of the 140 µg/kg-exposed hives observed in the feeding study, but not predicted by our model. Although these effects may disappear by 11 weeks after exposure, increased colony disease burden (from decreased immunity) and decreased food stores (from altered foraging behavior) could lead to colony failures in the fall or winter (Higes et al. 2008, vanEngelsdorp et al. 2009, Naug 2009). Interestingly, hives in the feeding study had a low number of workers, relative to pupae, across all treatments and time points. This low worker population, combined with clothianidin ingestion-induced mortality and possible sublethal effects at 140 µg/kg, may have pushed hives into failure. The VarroaPop model could better fit these scenarios by simulating pathogens in addition to *Varroa* mites; by including pesticide effects on immunity; and by adding feedback pathways critical to colony success such as thermoregulation, brood-rearing and hive defense capacity (Winston 1987, Stabentheiner et al. 2010, Barron 2015).

*Using a colony dynamics model to assess pesticide risk to bees*

Fitting a honey bee colony dynamics model to field-based experimental data allowed us to gain additional insights that could be leveraged as part of the risk assessment process used by regulatory agencies. Two key findings relative to the empirical colony feeding study were that some colony endpoints decline at lower exposure levels than our model predicted, and actual recovery of colony endpoints at the highest exposure level was less than our model predicted. As discussed above, these findings point to effects beyond oral toxicity-induced mortality,

504 suggesting that pathogen pressures and environmental variability also play important roles in
505 colony-level honey bee population dynamics.

506 We were also able to infer dose-response relationships from the empirical data, endpoints
507 which are typically difficult to estimate in whole-colony studies without a predictive model that
508 simulates internal hive processes. Our parameterized Bayesian model indicated that the median
509 oral $LD_{50}$ for adult bees in the feeding study was 16.3 ng/bee, which falls just above the range
510 observed in laboratory acute oral toxicity studies on individual bees, 2.6–15.7 ng/bee (Laurino et
511 al. 2011, USEPA 2017), and within the 95% confidence interval of one of the two registrant-
512 submitted studies (USEPA 2017). Interestingly, this suggests that Tier 1 individual bee toxicity
513 experiments with clothianidin could be informative for adult oral toxicity in ecologically-
514 relevant scenarios, despite their inherent simplicity. In contrast, our analysis of the feeding study
515 data provided little insight into the larval oral $LD_{50}$, as evidenced by the broad probability
516 distribution for this parameter which did not improve as ABC-SMC progressed, and there are no
517 other studies that directly assessed acute larval toxicity (USEPA 2017). This highlights one
518 drawback to highly parameterized inference methods: a dataset can lack sufficient information to
519 describe all parameters in a model (Luo et al. 2009). This issue of non-identifiability can occur
520 when parameters have functional interrelationships (correlation) (Li and Vu 2013), as in the case
521 of larval and adult toxicity, where larval mortality leads to fewer adults and adult mortality leads
522 to fewer larvae through reduced queen egg-laying rate. In fact, the feedback of adult mortality on
523 number of larvae may be responsible for the consistent and significant adverse effects predicted
524 for larvae at 72 and 140 µg/kg despite the wide range of possible larval $LD_{50}$ values. We
525 calculated fit to the empirical data based on the number of adults and eggs, but not larvae and

526 pupae, however, and including these latter two endpoints in future analyses may allow better
527 inference on larval toxicity.

528 We also leveraged our model to predict a more precise LOAEC for number of adults,
529 pupae, larvae and eggs by fitting toxicity parameters from the concentrations tested in the
530 feeding study, then predicting untested concentrations between 36 and 72 µg/kg, our initial
531 estimates of NOAEC and LOAEC. This method is more rigorous than simply interpolating
532 responses between two tested concentrations because it can account for uncertainty and non-
533 linear effects or tipping points, and it allows for consideration of statistical significance. We
534 estimated a more precise NOAEC and LOAEC of 55 and 60 µg/kg, respectively, based on
535 adverse effects across all endpoints. By comparison, a statistical analysis of the feeding study
536 data found a NOAEC and LOAEC of 19 and 36 µg/kg, respectively, for adults and pupae and 36
537 and 72 µg/kg, respectively for eggs and larvae (Louque 2016). It is important to remember that
538 our model-derived LOAEC describes the lowest concentration at which ingestion-induced
539 mortality is expected to begin significantly impacting colony-level endpoints but does not
540 consider other types of effects such as non-lethal effects or contact exposure that may be better
541 represented by the empirically-derived endpoints. Despite this caveat, our analysis shows how
542 colony dynamics models can estimate outcomes at exposure levels that could not be tested due to
543 logistical or financial constraints.

544

545 **Conclusion**

546 We challenged the VarroaPop+Pesticide bee colony dynamics model to simulate a publicly
547 available registrant-submitted dataset from a colony feeding study in which colonies were
548 exposed to pesticide-spiked nectar at six concentrations, and population-level effects were

549 tracked over several months. We successfully fit the model to these data using Approximate
550 Bayesian Computation with Sequential Monte Carlo, and inferred parameter distributions that
551 best describe the dose-response relationships and other key colony characteristics. Our results
552 demonstrate that honey bee colony models, combined with Bayesian model inference, can
553 investigate hypotheses about individual-level responses to pesticides from ecologically-relevant
554 colony-level data. These parameterized models can also predict how colonies will respond to
555 hypothetical scenarios such as untested concentrations, changes in weather or additional
556 stressors. Our findings suggest that applied colony dynamics models are a promising tool for
557 inference in support of higher-tier pesticide risk assessments.
558
559 **Acknowledgements and Disclaimer**
560 We thank Fran Rauschenberg (Senior Service America, Inc.), Sumathy Sinnathamby, Matthew
561 Etterson and John Wambaugh for manuscript review and edits. This research was supported in
562 part by an appointment to the Research Program at the National Exposure Research Laboratory
563 in the Office of Research Development, U.S. Environmental Protection Agency, administered by
564 the Oak Ridge Institute for Science and Education through Interagency Agreement No.
565 DW8992298301 between the United States Department of Energy and the United States
566 Environmental Protection Agency. This paper has been reviewed in accordance with Agency
567 policy and approved for publication. The views expressed in this article are those of the authors
568 and do not necessarily represent the views or policies of the USEPA. Any mention of trade
569 names, products, or services does not imply an endorsement by the USEPA.
570

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765

## Tables

**Table 1:** List of parameters considered to be random variables and inferred through Approximate Bayesian Computation.

| Parameter name in VarroaPop | Description | Units | Type | Lower limit | Upper limit |
|---|---|---|---|---|---|
| ICAdultLD50 | Oral $LD_{50}$ for adults | ng/bee | Prior | 0.1 | 100 |
| ICAdultSlope | Slope of the adult dose-response curve | % mortality/ng | Prior | 1 | 9 |
| ICLarvaLD50 | Oral $LD_{50}$ for larvae | ng/bee | Prior | 0.1 | 100 |
| ICLarvaSlope | Slope of the larva dose-response curve | % mortality/ng | Prior | 1 | 9 |
| ICForagerLifespan (mean) | Mean lifespan of foragers | days | Hyper prior | 4 | 16 |
| ICForagerLifespan (sd) | Std. dev. of forager lifespan | days | Hyper prior | 0 | 3 |
| ICQueenStrength (mean) | Mean queen strength ($\propto$ egg laying rate) | unitless | Hyper prior | 1 | 5 |
| ICQueenStrength (sd) | Std. dev. of queen strength ($\propto$ egg laying rate) | unitless | Hyper prior | 0 | 2 |

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**Table 2:** Percent of the study period that each treatment was predicted by our model to have a significant reduction in bee counts, compared to the control. The study period is the first day of treatment until the final colony condition assessment of 2014. An endpoint was considered significantly reduced (compared to the control) when the 95% prediction interval of the change did not contain zero. Clothianidin levels 50–70 µg/kg were predicted by the model but were not present in the empirical feeding study.

| | Percent of Study Period Significantly Lower Than Control | | | | |
|---|---|---|---|---|---|
| **Clothianidin exposure** | **Adults** | **Pupae** | **Larvae** | **Eggs** | **Mean** |
| **10 µg/kg** | 1.7 | 0.0 | 0.0 | 0.0 | **0.4** |
| **19 µg/kg** | 0.0 | 0.0 | 0.0 | 0.0 | **0.0** |
| **36 µg/kg** | 0.0 | 0.0 | 0.0 | 0.0 | **0.0** |
| *50 µg/kg* | 0.0 | 0.0 | 0.0 | 0.0 | **0.0** |
| *55 µg/kg* | 0.0 | 2.6 | 6.8 | 1.7 | **2.8** |
| *60 µg/kg* | 35.9 | 32.5 | 33.3 | 32.5 | **33.6** |
| *65 µg/kg* | 39.3 | 41.9 | 42.7 | 41.0 | **41.2** |
| *70 µg/kg* | 44.4 | 44.4 | 45.3 | 43.6 | **44.4** |
| **72 µg/kg** | 47.0 | 47.0 | 46.2 | 47.0 | **46.8** |
| **140 µg/kg** | 91.5 | 88.9 | 87.2 | 87.2 | **88.7** |

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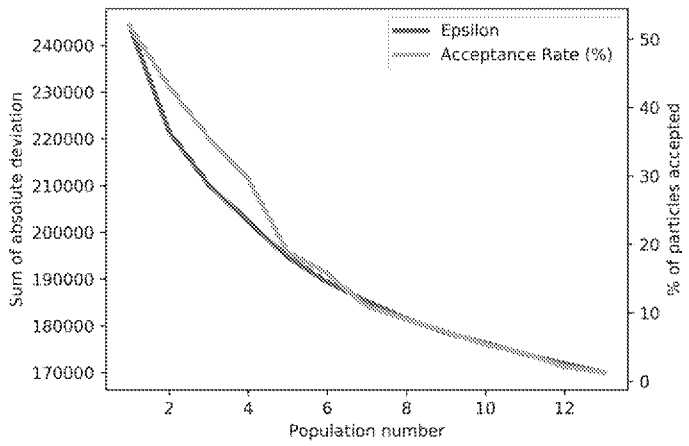
780 **Figures**

781




782

783 **Figure 1**: The acceptance threshold □ (blue) and acceptance rate (yellow) of Approximate

784 Bayesian Computation with Sequential Monte Carlo sampling through 13 populations.

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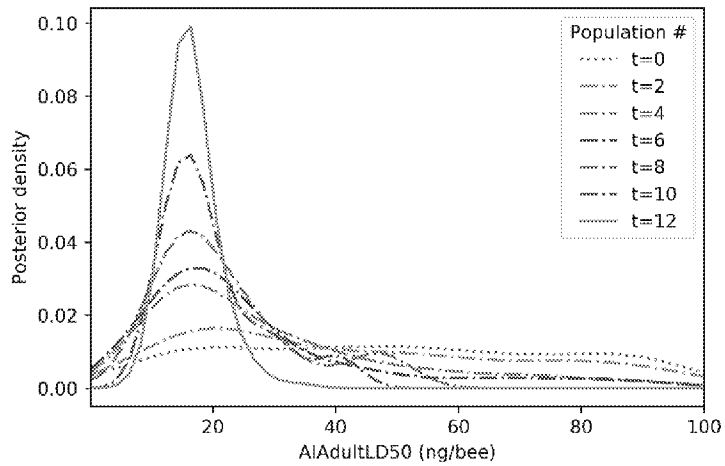


786

787 **Figure 2:** The posterior probability density of the adult oral $LD_{50}$ parameter through 13

788 generations (t) of Approximate Bayesian Computation with Sequential Monte Carlo sampling.

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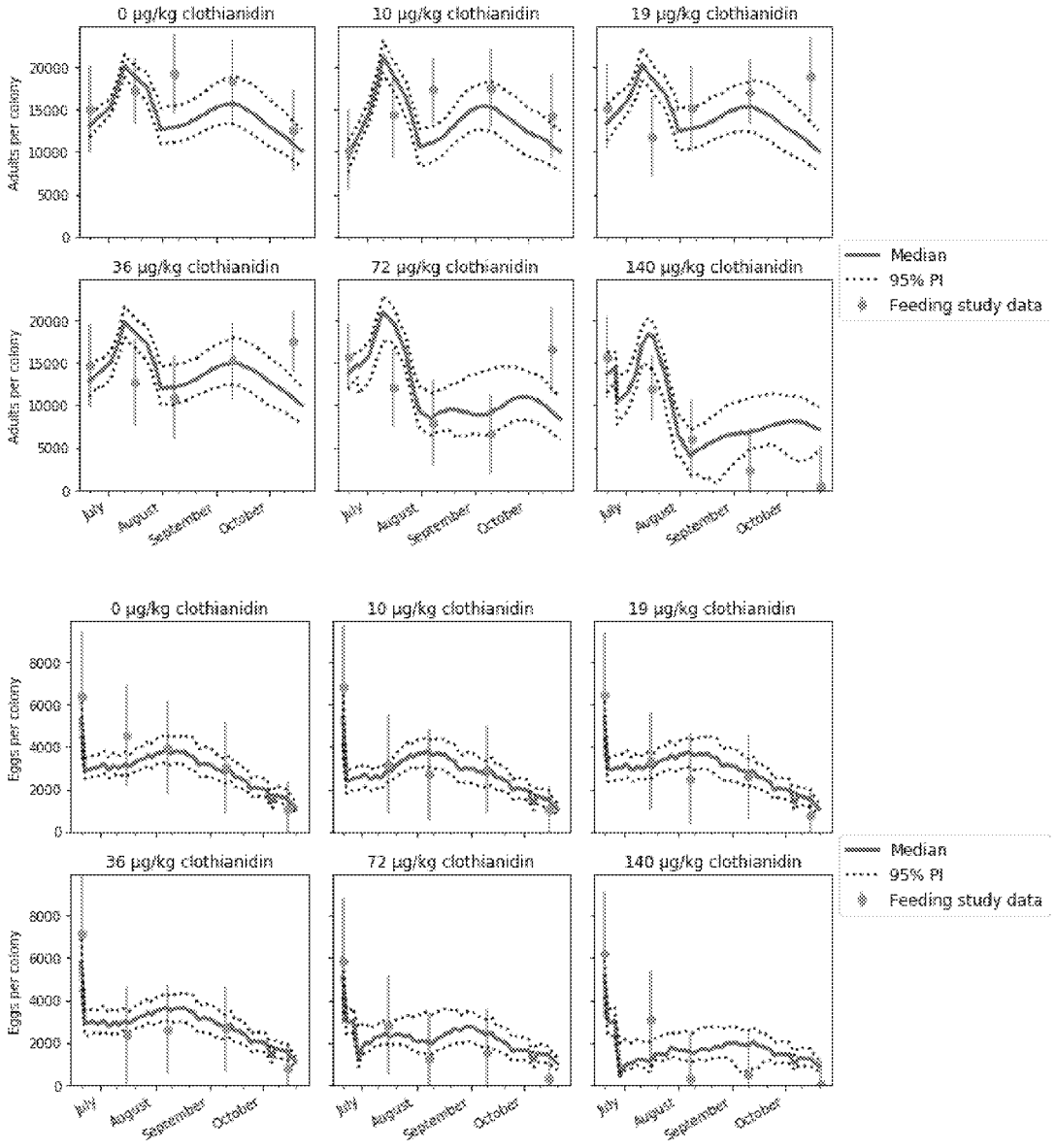


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791

792 **Figure 3**: Predicted number of adults (top) and eggs (bottom) during the feeding study versus
793 empirical data (orange dots with lines showing standard deviation). Solid blue lines represent the
794 median prediction and dotted blue lines denote the 95% prediction interval. For pupae and larvae
795 endpoints, see Appendix 2, Figure S2.

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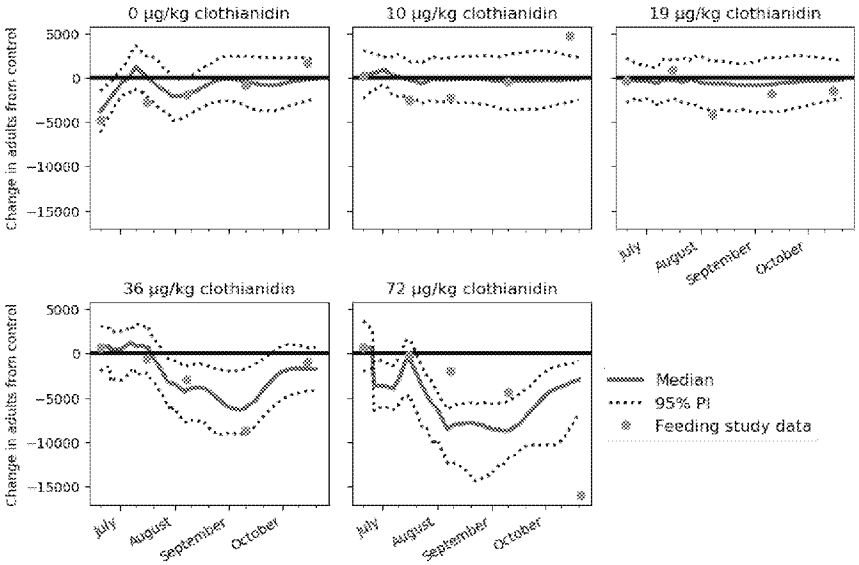


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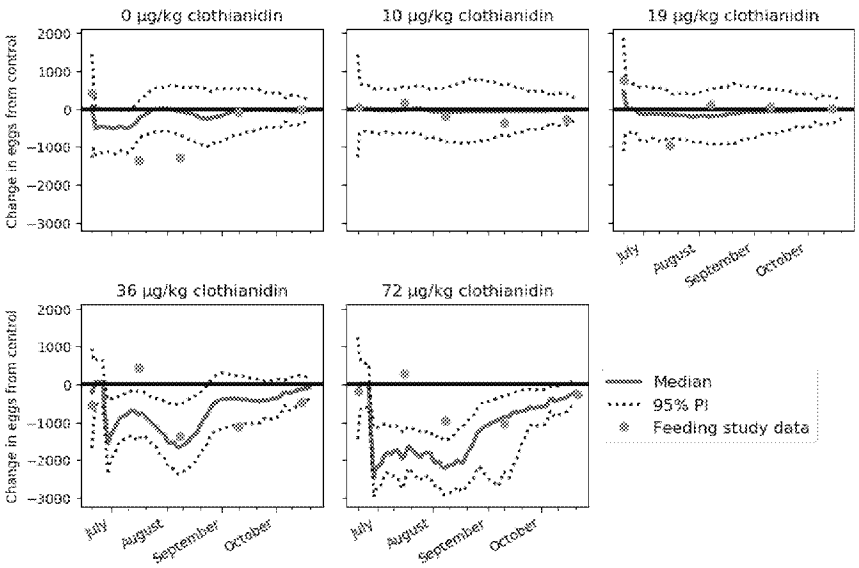


799

800 **Figure 4:** Predicted change in number of adults (top) and eggs (bottom) from the control.
801 Empirical feeding study data is represented by orange dots. Solid blue lines represent the median
802 prediction and dotted blue lines denote the 95% prediction interval. For pupae and larvae
803 endpoints, see Appendix 2, Figure S3.

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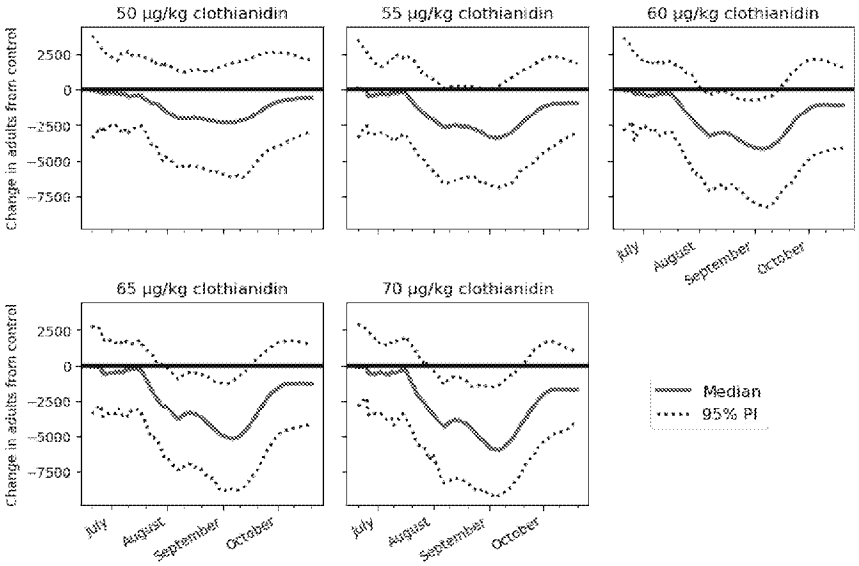


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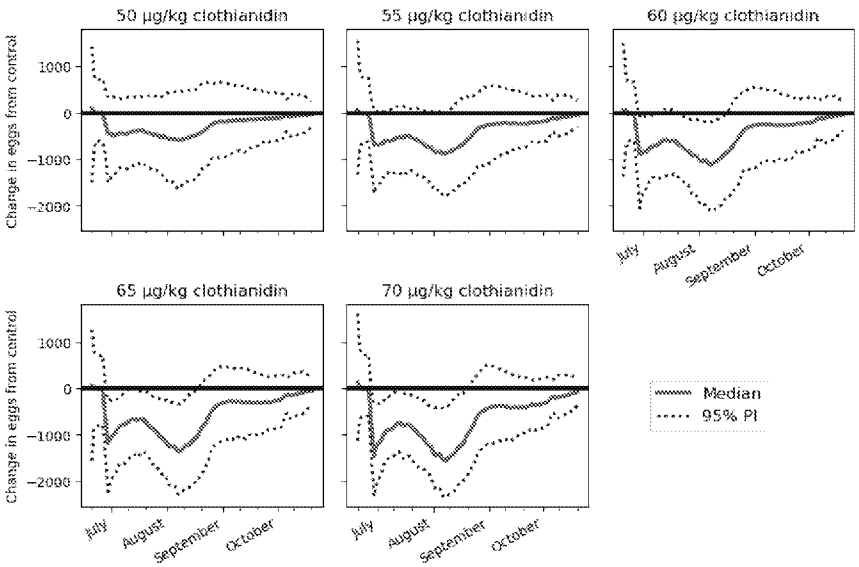


807

808 **Figure 5:** Predicted change, from the control, in number of adults for clothianidin levels not
809 tested in the feeding study. Solid blue lines represent the median prediction and dotted blue lines
810 denote the 95% prediction interval. For pupae and larvae endpoints, see Appendix 2, Figure S4.

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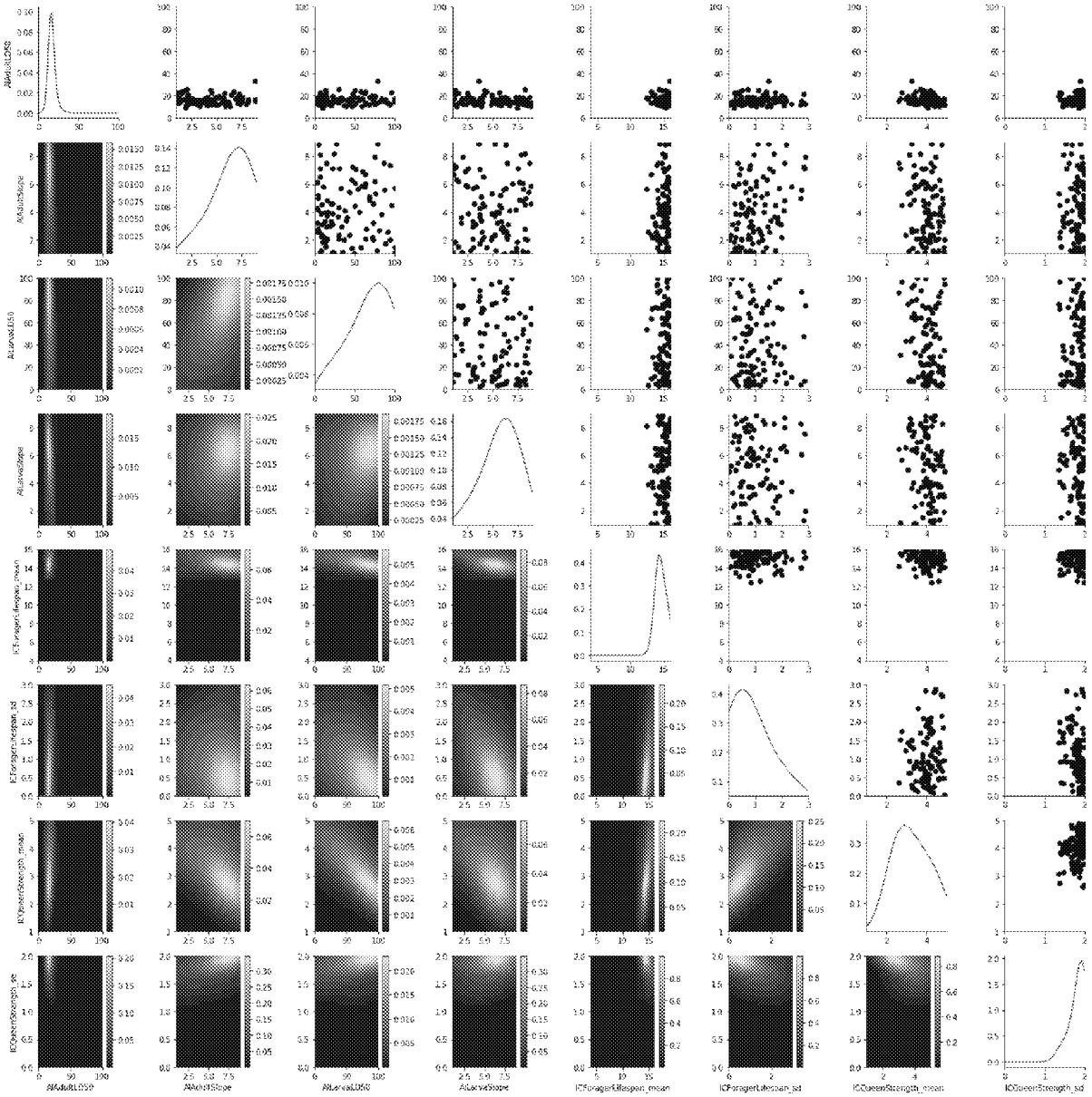


**Figure 6:** Posterior parameter distributions for our VarroaPop+Pesticide-based model, as inferred from the empirical feeding study data. Axes are labeled with parameter names specified in the VarroaPop+Pesticide program (see Table 1 for definitions). Marginal posterior distributions are shown in the diagonal. All priors were uniform across the x-axis range. Bivariate scatterplots (top half) and heatmaps (bottom half) of the distribution of accepted parameters illustrates covariance between parameters.

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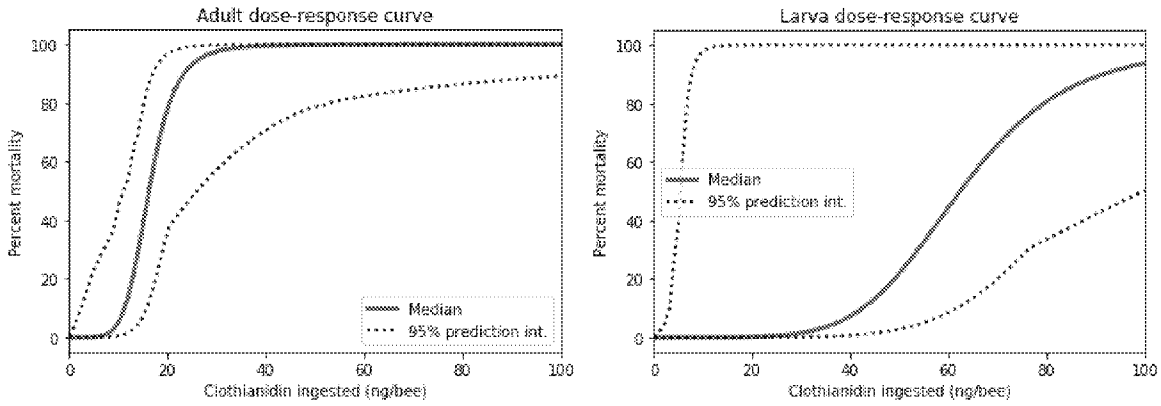


820

821 **Figure 7:** Our model's predicted adult (left) and larva (right) dose-response curves, given the
822 empirical feeding study data. Solid blue lines represent the median prediction and dotted blue
823 lines denote the 95% prediction interval.

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825 **Appendix 1**

826

827 **Table S1:** Static VarroaPop+Pesticide parameter values used in this study.

| Category | Parameter name | Description | Units | Value |
|---|---|---|---|---|
| *Foraging* | | | | |
| | IPollenTrips | Pollen trips per day | times/day | 8 |
| | INectarTrips | Nectar trips per day | times/day | 17 |
| | IPollenLoad | Pollen collected per trip | mg | 15 |
| | INectarLoad | Nectar collected per trip | mg | 30 |
| | ForagerMaxProp | Max. proportion of workers that can be active foragers | | 0.3 |
| *Consumption* | | | | |
| | CL4Pollen | Pollen consumption, worker larvae, age 4 days | mg/day | 1.8 |
| | CL5Pollen | Pollen consumption, worker larvae, age 5 days | mg/day | 3.6 |
| | CA13Pollen | Pollen consumption, worker adults, days 1–3 | mg/day | 6.7 |
| | CA410Pollen | Pollen consumption, worker adults, days 4–10 | mg/day | 6.7 |
| | CA1120Pollen | Pollen consumption, worker adults, days 11–20 | mg/day | 1.7 |
| | CForagerPollen | Pollen consumption, foragers | mg/day | 0 |
| | CLDPollen | Pollen consumption, drone larvae | mg/day | 3.6 |

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| | | | | |
|---|---|---|---|---|
| | CADPollen | Pollen consumption, drone adults | mg/day | 2.0 |
| | CL4Nectar | Nectar consumption, worker larvae, age 4 days | mg/day | 60 |
| | CL5Nectar | Nectar consumption, worker larvae, age 5 days | mg/day | 120 |
| | CA13Nectar | Nectar consumption, worker adults, days 1–3 | mg/day | 60 |
| | CA410Nectar | Nectar consumption, worker adults, days 4–10 | mg/day | 140 |
| | CA1120Nectar | Nectar consumption, worker adults, days 11–20 | mg/day | 60 |
| | CForagerNectar | Nectar consumption, foragers | mg/day | 292 |
| | CLDNectar | Nectar consumption, drone larvae | mg/day | 130 |
| | CADNectar | Nectar consumption, drone adults | mg/day | 225 |
| *Demographics* | | | | |
| | RQEnableReQueen | Enable requeening? | | off |
| | EToLXition | % of eggs that successfully transition to larvae | % | 100 |
| | LToBXition | % of larvae that successfully transition to pupae (brood) | % | 100 |
| | BToAXition | % of pupae (brood) that successfully transition to adults | % | 60 |
| | AToFXition | % of adults that successfully transition to foragers | % | 100 |

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## Appendix 2

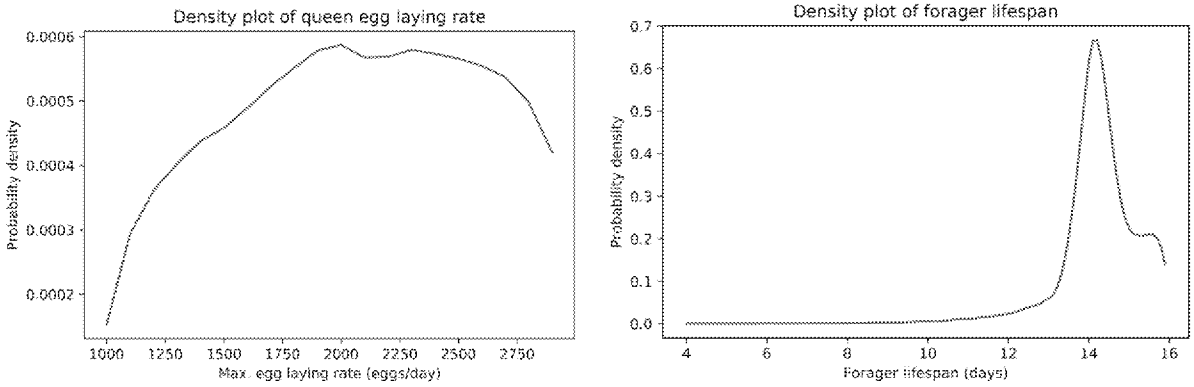


**Figure S1:** Probability density of maximum queen egg laying rate (eggs/day) and forager lifespan (days) for individual colonies in the feeding study, as predicted from 200 draws of the joint posterior.

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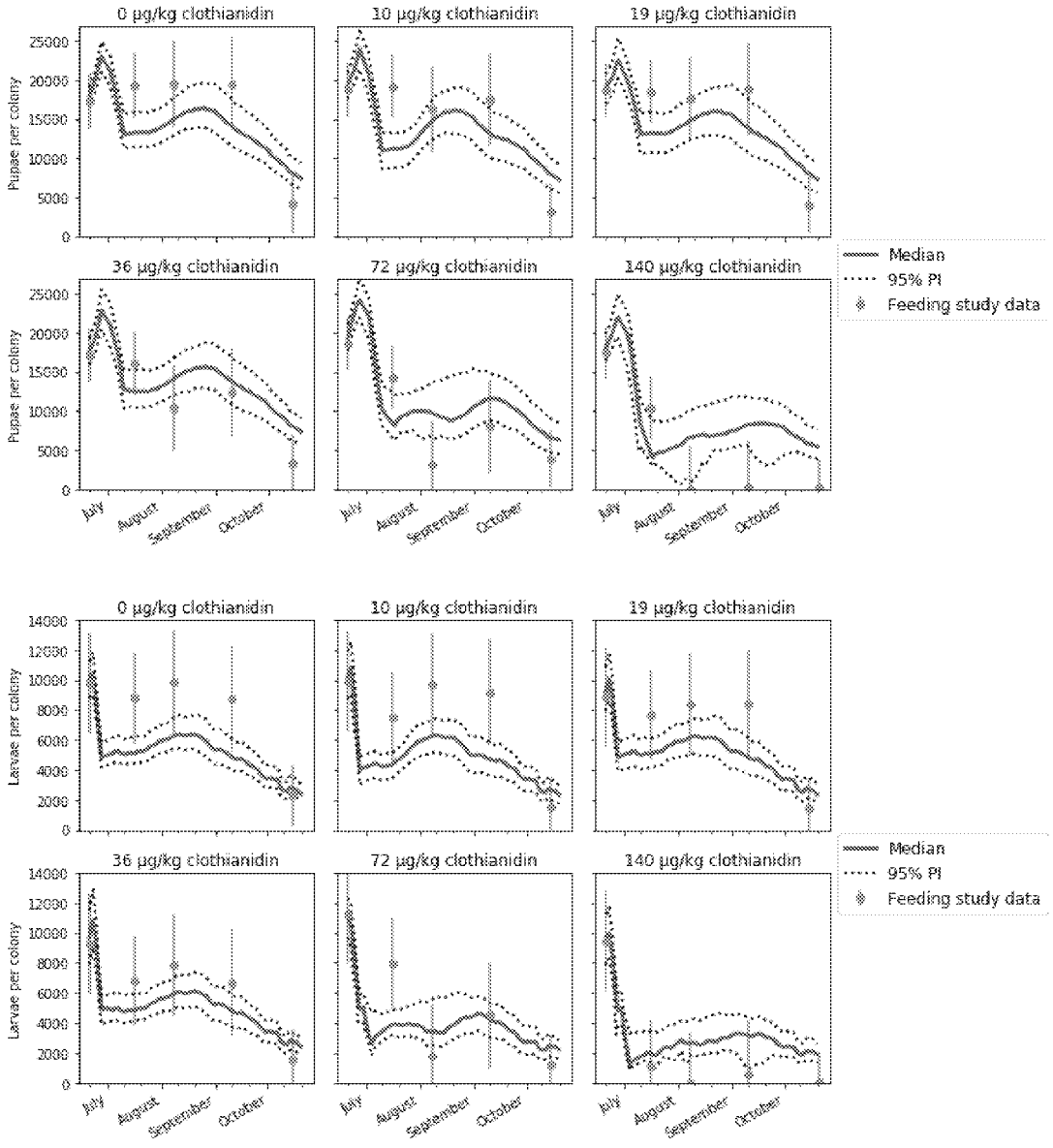


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840 **Figure S2:** Predicted number of pupae (top) and larvae (bottom) during the feeding study versus
841 the empirical data (orange dots with lines showing standard deviation). Solid blue lines represent
842 the median prediction and dotted blue lines denote the 95% prediction interval.

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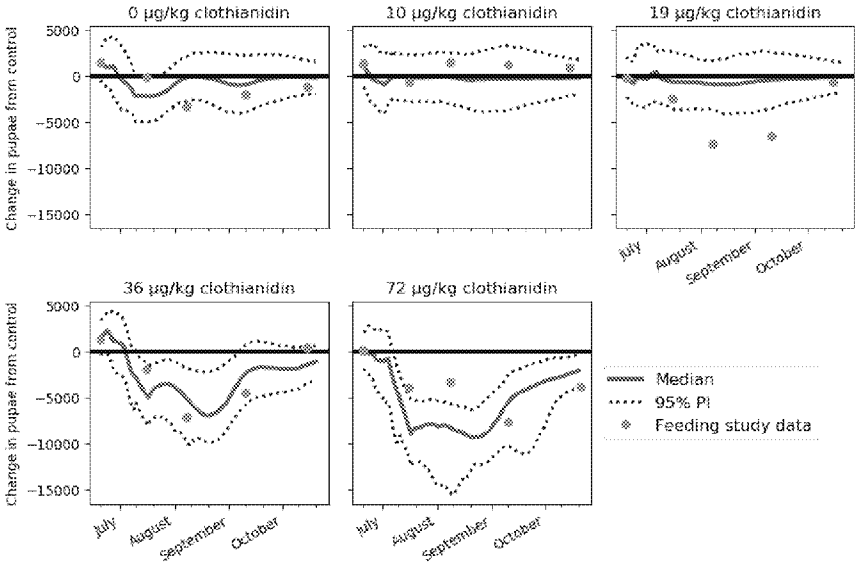


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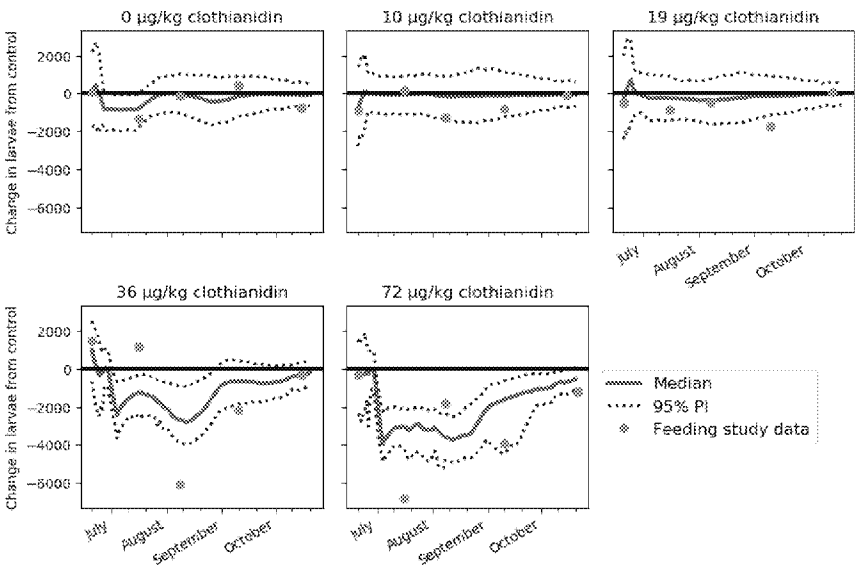


845

846 **Figure S3:** Predicted change in number of pupae (top) and larvae (bottom) from the control.

847 Empirical feeding study data is represented by orange dots. Solid blue lines represent the median

848 prediction and dotted blue lines denote the 95% prediction interval.

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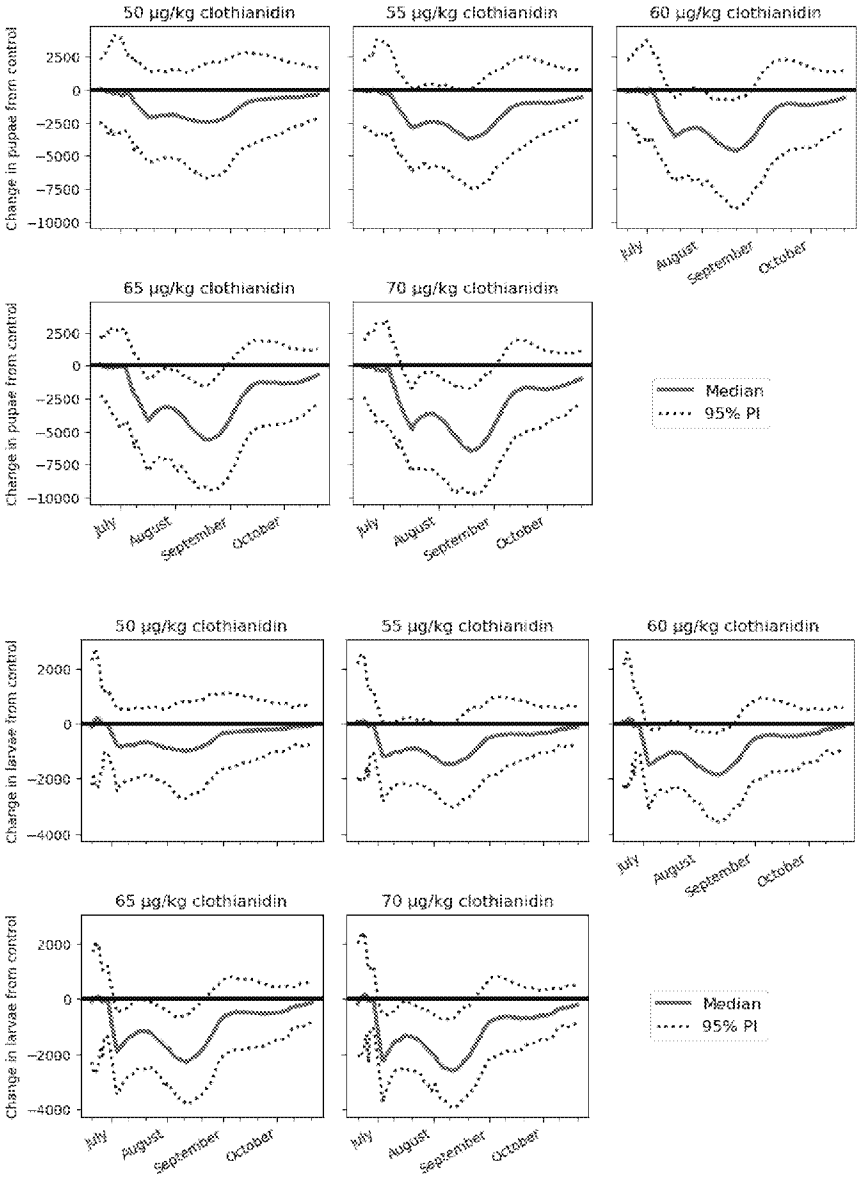


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851

852 **Figure S3:** Predicted change, from the control, in number of adults for clothianidin levels not

853 tested in the feeding study. Solid blue lines represent the median prediction and dotted blue lines

854 denote the 95% prediction interval.

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